Title: Prevention, therapy and prognosis/monitoring in
sepsis and septic shock

FIELD OF THE INVENTION

The invention is in the field of medicine, more
particularly the field of prognosis, prevention and therapy
5   of sepsis and septic shock. The invention provides means
for therapeutic prevention and treatment of sepsis and
septic shock, as well as means to determine the severity of
a septic condition and to make a prognosis for the further
course, and means for septic monitoring of the course of a
10   treatment of sepsis and septic shock.

BACKGROUND OF THE INVENTION

Sepsis and septic shock.
15   A normal bacterial inflammation is a highly
regulated process whereby the defence system clears up
bacteria. In humans with a weakened defence, the bacterium
gets an opportunity to proliferate (virtually
uninhibitedly) and to break through to the blood stream.
20   The presence of bacteria in the blood stream is called
sepsis. Sepsis leads to the production of proinflammatory
mediators by white blood cells (monocytes and macrophages)
in the blood. This reaction is crucial for a fast and
efficient elimination of bacteria. However, if the
25   inflammatory reaction persists too long, for instance due
to disease-related immune suppression, this may eventually
lead to severe fever, spontaneous blood clotting and damage
of tissues due to oxygen deficiency and due to bactericidal
products. In serious cases, organs (specifically kidney,

heart and liver) may stop functioning and the patient may
get into so-called septic shock, from which he may
subsequently die.

5   *Incidence.*

         In the United States alone, more than 500,000
patients a year develop septic shock, with an estimated
incidence increase of about 1.5% a year, and the costs
amount to $16.7 billion a year (Angus, 2001). Septic shock
10   results in death in 20-80% of cases (depending on the
definition used). The increasing use of invasive surgical
techniques, chemotherapy and the application of
immunosuppression in patients with organ transplants or
inflammatory diseases constitute causes of an increasing
15   number of cases of sepsis. The longer life expectancy of
elderly people and patients having metabolic, neoplastic
and immunodeficiency defects owing to improved medical
care, also leads to populations at increased risk of
bacterial infection.
20

Lipopolysaccharide and lipoteichoic acid.

         Sepsis and septic shock are caused chiefly by Gram-
negative bacteria (for instance *E. coli* and *K. pneumoniae*)
and (to a lesser extent) Gram-positive bacteria (*S. aureus*
25   and *S. epidermidis*).

         Lipopolysaccharide (LPS) is the most important
constituent of the outer membrane of Gram-negative
bacteria. LPS is the most reactive component of Gram-
negative bacteria. LPS activates mononuclear cells
30   (monocytes and macrophages), after which these cells
produce proinflammatory mediators such as cytokines (TNFα,

IL-1ß, IL-6) and oxygen radicals. This response is
necessary for a fast and effective dealing with the
bacterial infection, and plays a crucial role in the
clearing up of the bacteria (Heumann, 2002). However, in
5   case of an inadequate response, allowing the bacteria to
continue to proliferate uninhibitedly, these mediators are
responsible for the metabolic changes that lead to
pathologic conditions and eventually to death, as
demonstrated in rabbits, monkeys and mice.
10   The equivalent of LPS in Gram-positive bacteria is
lipoteichoic acid (LTA), which possesses a structure that
exhibits strong homology to that of LPS.

Current clinical and experimental therapies.

15   The complexity of sepsis and the immunological
defense render the development of pharmacological
interventions more difficult. The standard treatment often
consists in the administration of fluid and blood pressure
raisers (so-called vasopressors) to normalize the blood
20   pressure and the oxygen supply of organs, and antibiotics
to inhibit the proliferation of bacteria (Rackow, 1991;
Cohen, 1991; Wheeler, 1999), but this treatment is not
sufficient to prevent the high mortality.
        Most applied experimental strategies for treating
25   sepsis have been aimed at inhibiting the proinflammatory
response, for instance through capturing LPS, blocking the
cytokine action or overall immune suppression.
        However, various clinical studies have shown that
LPS-neutralizing antisera are not effective in septic shock
30   (Cohen, 1999). Likewise, experimental therapies with
antibodies to inter alia TNFα (Fisher, 1996; Clark, 1998)

and the IL-1 receptor (Fisher, 1994; Opal, 1997) proved not to be effective.

By means of a meta-analysis, it has been demonstrated that overall immune suppression increases the

5 mortality resulting from sepsis (Cronin, 1995). A recent study in humans demonstrates that a polymorphism in the LPS receptor Toll-like receptor 4 (TLR4), which leads to a reduced inflammatory response against LPS, is associated with an increased sensitivity to severe bacterial infection

10 (Kiechl, 2002). Furthermore, it is known that mice, which are deficient for the proinflammatory cytokine macrophage migration inhibitory factor (MIF), are less well capable of clearing up bacteria such as *Leishmania major* (Satoskar, 2001) and *Salmonella typhimurium* (Koebernick, 2002).

15 Many suggestions have been made for treating sepsis or septic shock, all of which have in common that an LPS-binding peptide is administered to sepsis patients. The administration of these LPS binding peptides is typically aimed at the suppression of the LPS-induced activation of

20 the immune system. The peptides to be administered can, for instance, be derived from LBP (LPS Binding Protein; see WO 95/25117), apoA and apoE (WO 98/07751), apoA1 (WO 95/25786; WO 99/16458; WO 99/16459; WO 99/16408; WO 99/16409), CAP18 (Larrick, 1994), CAP37 (US 6107460; US 5650392; US 5627262;

25 US 5607916), CD14 (WO 96/20956), prophenin (WO 95/34289; WO 95/26747), polyphemusin (WO 02/00687) and LALF protein (US 5747455). To date, however, all of these suggestions have not yielded an effective approach for sepsis and septic shock either.

30 Sepsis has great consequences for the lipoprotein metabolism. Patients who get to the clinic exhibit strongly

lowered cholesterol levels in the blood, which normalize after successful antibacterial therapy. Research *in vitro* and in experimental animals has demonstrated that lipoproteins can offer protection against sepsis, but the

5   complexity of lipoproteins (including composition, endogenous character) makes their clinical application more difficult. Also, the mechanism by which lipoproteins play a protective role is still unknown. It has been reported that infusion of protein-free triglyceride-rich lipid emulsions

10   does not afford protection (Van der Poll, 1995), which indicates that it is precisely the protein components of lipoproteins that can play a large protective role in sepsis.

15   **BRIEF DESCRIPTION OF THE INVENTION**

An object of the invention is to provide a prevention or effective handling (therapeutic treatment) of sepsis and septic shock, which is more efficient than the

20   treatment methods proposed heretofore and/or avoids disadvantages thereof.

Another object of the invention is to provide means and methods by which the proinflammatory reaction as induced by the toxic components of bacteria, in particular

25   LPS and/or LTA, in septic patients can be enhanced.

A further object of the invention is to provide a reliable determination of the severity of a septic condition and reliable prognosis of sepsis or septic shock.

A still further object of the invention is to

30   provide a reliable evaluation of the pathologic condition

of septic patients, so that the effect of a treatment of
the disease can be measured. .

     To that end, the invention provides the use of a
peptide which binds to lipopolysaccharide (LPS) or
5   lipoteichoic acid (LTA), for manufacturing a pharmaceutical
composition for treating sepsis or septic shock, the
peptide comprising the amino acid sequence of
apolipoprotein CI (apoCI) or a part thereof that comprises
at least the amino acids of the C-terminal helix of apoCI.
10   In particular, this concerns a peptide which
comprises the amino acid sequence of human apoCI or a part
thereof that comprises at least the amino acids of the
C-terminal helix of human apoCI. However, apoCI of a non-
human mammal, or a corresponding fragment thereof, can also
15   be used. For applications in, e.g., domestic animals, such
as horse, cow, dog and cat, typically the use of an apoCI
of the respective mammal will be preferred.

     Most preferred according to the invention, however,
is that the peptide is human apoCI or a fragment thereof
20   that comprises at least the amino acids MREWFSETFQKVKEKLK.

     Concretely, the peptide is preferably human apoCI having
the amino acid sequence TPDVSSALDKLKEFGNTLEDKARELISRIKQSE
LSAKMREWFSETFQKVKEKLKIDS. However, it is also possible to use a
fragment, for instance the peptide SAKMREWFSETFQKVKEKLKIDS or
25   the peptide MREWFSETFQKVKEKLK.

     In particular, this concerns the use of a peptide
which binds to lipopolysaccharide (LPS), as defined herein,
whereby a pharmaceutical composition is manufactured for
preventing or treating a sepsis or septic shock caused by
30   Gram-negative bacteria in mammals, in particular humans or
a domestic animal, such as horse, cow, dog and cat.

However, the invention also comprises the use of a
peptide that binds to lipoteichoic acid (LTA), as defined
herein, whereby a pharmaceutical composition is
manufactured for preventing or treating a sepsis or septic

5   shock caused by Gram-positive bacteria in mammals, in
particular humans or a domestic animal, such as horse, cow,
dog and cat.

Also, according to the invention, there is provided
a pharmaceutical composition for preventing or treating

10   sepsis or septic shock, which composition comprises a
peptide as defined herein, as well as a pharmaceutically
acceptable carrier.

Further, the invention provides a method for
preventively treating a mammal, in particular human

15   individual, which, for instance as a result of a surgical
intervention or a weakened or deficient immune system, is
at increased risk of developing sepsis, wherein an active
amount of a peptide as defined herein is administered to
the mammal.

20   Likewise, the invention provides a method for
treating a mammal, in particular human individual, which
suffers from sepsis or septic shock, wherein an active
amount of a peptide as defined herein is administered to
the mammal.

25   Also, the invention provides a method for
determining the severity of a septic condition and making a
prognosis for the further course of the sepsis or septic
shock in a mammal, in particular human individual, which is
suffering from sepsis or septic shock, wherein the apoCI

30   content in a blood sample of the mammal is determined.

Likewise, the invention provides a method for
monitoring a treatment of sepsis or septic shock in a
mammal, in particular human individual, which is being
treated for sepsis or septic shock, wherein the apoCI
5   content in a blood sample of the mammal is determined.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the relation between the apoCI level
10   in the blood plasma of sepsis patients and the lapse of
time after admission to the clinic. The figure demonstrates
the predictive value of the apoCI level for the chance of
survival of sepsis.
Figure 2 shows the effect of an electrophoretic
15   field on the migration of apoCI and/or LPS through an
agarose matrix. The figure demonstrates a physical
interaction between apoCI and LPS, which leads to
comminution of LPS aggregates and also to reduced
recognition of apoCI by a specific antibody.
20   **Figure 3** shows the relation between the molar
LPS:apoCI ratio and the inhibition of the stain reaction
during ELISA by which the concentration of apoCI is
determined. The figure shows a strong interaction between
apoCI and LPS, which can prevent the binding of a specific
25   antibody to apoCI.
Figure 4 shows the effect of apoCI and apoCIII on
the time-dependent deaggregation of LPS. The figure shows a
dose-dependent monomerization of LPS micelles by apoCI,
while apoCIII is hardly effective.
30   **Figure 5** shows the effect of the apoCI concentration
in murine plasma on the time-dependent monomerization of

LPS. The figure shows that the absence of apoCI strongly
inhibits the monomerization of LPS, while expression of
human apoCI increases the monomerization of LPS dose-
dependently.

5   **Figure 6** shows the effect of apoCI and bovine serum
albumin on the behavior of radioactively labeled LPS after
intravenous injection in mice. The figure shows that apoCI
is capable of strongly reducing the uptake of LPS by the
liver, while the residence time of LPS in the blood

10   increases considerably through the apoCI-induced binding of
LPS to HDL. Bovine serum albumin, by contrast, proved not
effective.

           Figure 7 shows the effect of apoCI on the LPS-
induced proinflammatory response. The figure shows that the

15   interaction of LPS with apoCI strongly increases the
inflammatory response (i.e. TNFα induction).

           Figure 8 shows the correlation between the apoCI
levels in the plasma of patients who develop endotoxemia
during a heart operation with cardiopulmonary bypass, with

20   pre- and post-operative TNFα levels. The figure shows that
there is a strongly significant positive relation between
the apoCI level and the TNFα level in patients who develop
endotoxemia, but not in patients who do not develop
endotoxemia. Such a relation could not be demonstrated for

25   apoCIII or apoE.

DETAILED DESCRIPTION OF THE INVENTION
Role of apoCI in sepsis.

           We have found, as Example 1 and Figure 1 show, that

30   the level (the concentration) of apolipoprotein CI (apoCI)
in septic blood is strongly lowered, which does not hold,

or holds to a much lesser extent, for the (numerous) other
apolipoproteins. This lowered apoCI content in septic blood
is confirmed by the literature (Barlage, 2001), although
the authors have not discussed a possible role of apoCI in
5   sepsis.

ApoCI is the smallest apolipoprotein (6.6 kDa, 57
amino acids) which is chiefly synthesized by the liver and
circulates in the blood in a fairly high concentration
(~6 mg/dL), both in free, unbound form and as a constituent
10   of lipoproteins (chylomicrons, VLDL, HDL) (Jong, 1999). The
amino acid sequence of apoCI is known (see Table 1). To
date, no mutations have become known that lead to different
variations ('polymorphisms') of apoCI. Using transgenic
mice that overexpress the human apoCI, we have demonstrated
15   that apoCI plays a role in the lipid metabolism, since
apoCI overexpression leads to increased lipid levels in the
blood (Jong, 1996; Jong, 1998), but a role, if any, of
apoCI in sepsis was not yet known.

We have developed the hypothesis that the apoCI
20   depletion associated with sepsis is a direct consequence of
bacterial infection. The low apoCI concentration might be
the consequence of binding of apoCI to bacterial products,
specifically LPS, which may possibly lead to accelerated
clearance of apoCI from the blood.

25   Via an extensive literature study, it was found that
LPS-binding proteins have common characteristics, with the
presence of a combination of positively charged and
hydrophobic amino acids being essential. Two of these
proteins are the limulus anti-LPS factor (LALF) (Hoess,
30   1993), a factor in the 'LAL assay' routinely used to

measure the LPS activity, and CAP18 (Larrick, 1994), which
is synthesized by polymorphonuclear cells.

In fact, apoCI contains a large number of positively
charged lysines (one in each six amino acids), alternated
5   with hydrophobic amino acids. To our surprise, human apoCI
contains a peptide sequence in its C-terminal portion
(**KV**KEK**L**K) which is virtually identical to the LPS-binding
sequences of LALF (**KW**KYK**G**K) and CAP18 (**K**I**KEKL**K), where
symbols in bold-type and italics respectively represent
10   positively charged and hydrophobic amino acids. We have
subsequently demonstrated that apoCI, both in pure form and
as a constituent of plasma in fact binds strongly to LPS
(see Example 2 and Figures 2, 3, 4 and 5).

We have compared the amino acid sequences of apoCI,
15   such as occur in man (SwissProt P02654), baboon (SwissProt
P34929), dog (SwissProt P56595), rat (SwissProt P19939) and
mouse (SwissProt P34928), as can be found under the numbers
indicated (http://www.expasy.ch/sprot/). See Table 1.

20   **Table 1. Primary amino acid sequences of apoCI.**

Human apoCI (1-57)

TPDVSSALDKLKEFGNTLEDKARELISRIKQSELSAKMREWFSETFQKVKEKLKIDS
Baboon apoCI (1-57)
25   APDVSSALDKLKEFGNTLEDKAWEVINRIKQSEFPAKTRDWFSETFRKVKEKLKINS
Dog apoCI (1-62)
AGEISSTFERIPDKLKEFGNTLEDKARAAIESIKKSDIPAKTRNWFSEAFKKVKEHLKTAFS
Rat apoCI (1-62)
APDFSSAMESLPDKLKEFGNTLEDKARAAIEHIKQKEIMIKTRNWFSETLNKMKEKLKTTFA
30   *Mouse apoCI (1-62)*
APDLSGTLESIPDKLKEFGNTLEDKARAAIEHIKQKEILTKTRAWFSEAFGKVKEKLKTTFS

The consensus LPS-binding sequence underlined in
Table 1 as found in human apoCI (KVKEKLK) has been found to
occur virtually unmodified in the animal variants. Human
apoCI forms two amphipatic helices of class 2 (amino acid
5   sequences 7-29 and 38-52), which facilitate the binding of
apoCI with lipoproteins. On the basis of structural
homology, it is assumed that animal apoCI takes a
comparable structure.

10   *Working mechanism of apoCI.*
On the basis of the disappointing results of the
earlier discussed, failing experimental antiseptic
therapies, we have furthermore developed the hypothesis
that therapeutic reduction of the proinflammatory response
15   is not only ineffective but even undesirable, because in
this way the causative bacterial proliferation is not
inhibited. Although the cause of sepsis is very
heterogeneous and the time of intervention may be
determinative of the success of the treatment, we deem it
20   likely that specifically enhancement of the inflammatory
response in an early stage of sepsis increases the
antibacterial effectiveness and hence raises the chance of
survival.
While proteins, such as CAP18, LALF, CAP37,
25   prophenin and polyphemusin, which do not form part of
lipoproteins, are also capable of binding LPS, they do not,
in contrast with apoCI, occur in a high concentration in
the blood. Further, these proteins lead to suppression of
the LPS-related inflammation cascade, which according to
30   our hypothesis is unfavorable for the course of the

disease. The bacterial infection will, as a result,
aggravate rather than improve.

   Surprisingly, we have now established that the
binding of LPS to human apoCI leads to a considerably
5   longer residence time of LPS in the blood (see Example 4
and Figure 6), owing to its binding to plasma lipoproteins,
facilitated by the amphiphilic structure of apoCI. Also, we
have established that administration of LPS with apoCI to
mice leads to a strongly increased proinflammatory response
10   compared with mice that were administered LPS alone, which
appears from a fourfold increased TNFα production (see
Example 5 and Figure 7). The finding that apoCI enters into
a direction interaction with LPS is new and points to a
direct role of apoCI in the endogenous control of bacterial
15   sepsis.

   Because apoCI is a constituent of lipoproteins
(chylomicrons, VLDL and HDL) and is consequently cleared
slowly from the blood, we therefore suppose that the
binding of LPS to apoCI extends the residence time of LPS
20   in the blood, so that the activation of white blood cells
is additionally stimulated in a natural way. As a result,
the production of proinflammatory cytokines (TNFα, IL-1ß,
and IL-6) will increase and consequently the antibacterial
inflammation cascade will work extra strongly. This
25   mechanism can also partly explain why lipoproteins play a
protective role in sepsis. Accordingly, the essence of the
present invention is that through administration of apoCI,
or an adequate fragment thereof, as defined herein, a
timely, extra forceful inflammation cascade is obtained
30   which enables early neutralization of both LPS and the LPS-

producing bacteria, so that the stage of full-blown sepsis
or septic shock will not be reached.

Clinical applicability.

5   The small size of apoCI enables obtainability of the
protein on a large scale through so-called solid-phase
peptide synthesis. This holds to a greater extent for
smaller peptides (fragments of apoCI), which bind LPS and
stimulate LPS-induced activation of white blood cells.

10   Since apoCI is an endogenous plasma protein, it will be
tolerated well and not generate any (additional) immune
reactions. (For that reason, use of human apoCI will be
preferred in humans, while in animals, preferably the
corresponding animal apoCI will be used).

15   It is noted that according to the invention, apoCI,
or a suitable fragment thereof, is used as the active
substance to control or prevent sepsis or septic shock.
According to the invention, the apoCI is not used as
carrier for another substance. According to the invention,

20   the apoCI, or fragment thereof, is preferably used as free
peptide, not bound in complexes with lipids.

Measurement of apoCI content for prognosis or monitoring.

As is demonstrated in Example 1 and Figure 1,
25   patients having severe sepsis exhibit a strongly lowered
apoCI level. In the example given (17 patients admitted to
the clinic with severe sepsis) the apoCI level was on
average 1.6 mg/dL. The apoCI levels in patients who
eventually survived the sepsis (47%: 8/17) exhibited a

30   progressive rise after 3 days and normalized after about 4
weeks. By contrast, the apoCI levels in the patients who
eventually died from the sepsis within 4 weeks (53%: 9/17),
did not exhibit any rise. These data show that monitoring
of the apoCI concentration in blood plasma has a prognostic

35   value for the chance of survival of the septic patient (see

also Figure 1), and can be used for monitoring the effect
of antiseptic therapies.

A. The peptides to be used according to the invention.

5   As set out above, the invention comprises the
application of apoCI and peptides derived therefrom which
bind to LPS for the therapeutic treatment of Gram-negative
bacterial sepsis. However, since apoCI and peptides derived
therefrom also bind to lipoteichoic acid (LTA), the toxic
10   equivalent of LPS in Gram-positive bacteria, the invention
comprises the use of apoCI in bacterial infections in
general, hence also in Gram-positive bacterial sepsis.

        According to a particular preferred embodiment, the
peptide consists of the whole amino acid sequence of apoCI
15   (or comprises this sequence), in particular that of human
apoCI, which consists of 57 amino acids according to the
amino acid sequence
TPDVSSALDKLKEFGNTLEDKARELISRIKQSELSAKMREWFSETFQKVKEKLKIDS.

        According to another preferred embodiment, the peptide
20   is, or comprises, the amino acid sequences of homologues of
human apoCI such as they occur in the animal kingdom (in
particular in mammals, for instance horse, cow, dog,
rabbit, rat, mouse).

        According to another preferred embodiment, the
25   peptide is, or comprises, at least the C-terminal helix of
apoCI. In human apoCI, the C-terminal helix is formed by
the amino acids 38-52, with the amino acid sequence
MREWFSETFQKVKEK. Preferably, a useful fragment of human
apoCI comprises at least the amino acids MREWFSETFQKVKEKLK.
30   Examples of suitable peptides are, for instance, the
peptides MREWFSETFQKVKEKLK and SAKMREWFSETFQKVKEKLKIDS.
These peptides comprise the C-terminal helix of apoCI and
the whole putative LPS binding sequence KVKEKLK. Instead of
a fragment of human apoCI, a corresponding fragment of an
35   animal apoCI can be used.

A property important according to the invention of
apoCI and the peptides defined herein, is, besides their
binding to LPS, their binding to lipoproteins.

5   **B. Obtaining the peptides.**

The complete human apoCI protein (amino acids 1-57)
is commercially available (BIODESIGN International, Maine,
USA and INTRACEL Corporation, Maryland, USA). Also,
procedures have been described to isolate human apoCI as
10   pure protein from blood (Tournier, 1984; Jackson, 1986).
Finally, there is the possibility of constructing an
adenoviral expression vector that codes for human or animal
apoCI, so that apoCI can be produced on a large scale by
astrocytoma cells in culture (Kypreos, 2001). Because apoCI
15   is a very small protein, there is also the possibility of
obtaining it from the individual amino acids by means of
solid phase peptide synthesis (Clark-Lewis, 1986). This
technique also enables synthesization of all human apoCI
analogs and other apoCI-derived peptides as described
20   herein.

C. Administration forms and routes.

Human apoCI is water-soluble and so can be
administered intravenously in an aqueous solution (for
25   instance in a physiological saline solution). Single-time
(bolus) injection is a possibility, as is continuous
infusion.

Also, apoCI has lipid-binding properties, which
further permits intravenous administration as a constituent
30   of commercially available triglyceride-rich lipid emulsions
(e.g. Intralipid®, Intrafat® and Lipofundin®) which are
clinically routinely used as efficient parenteral energy
source. In this case, too, bolus injection and continuous
infusion both are possibilities.

17

All this likewise holds for the peptides according to the invention.

Oral administration of peptides according to the invention and of apoCI in particular is in principle also
5 possible (Buclin, 2002).

Pharmaceutically acceptable carriers that are suitable for these different administration routes are naturally known to those skilled in the art.

10 **D. Therapeutic dose.**

The plasma concentration of apoCI in healthy individuals is on average 6 mg/dL (Curry, 1981), while we have demonstrated that apoCI in septic patients is strongly lowered. It is expected that a plasma concentration of
15 0.05-500 mg/dL (*preferably* 0.5-50 mg/dL) can be therapeutically effective.

Based on an average plasma volume of 2.5 L given a body weight of 70 kg, it can be calculated that a dose of 0.02 mg/kg-200 mg/kg (*preferably* 0.2 mg/kg-20 mg/kg) can be
20 effective in the case of a single-time (bolus) injection.

When administered as infusion, the dose will be strongly dependent on the rate of clearance of the apoCI from the blood, so that the dosing can vary within wide limits, for instance from 0.01 to 10 mg/kg/minute.
25

E. Expected effect of the treatment.

It is expected that apoCI will bind freely circulating LPS and LTA and will keep them present in the blood longer, so that LPS and LTA will be able to induce an
30 increased proinflammatory antibacterial reaction, so that bacteria and LPS are neutralized faster and more effectively. Both preventive application of apoCI in individuals at increased risk of developing sepsis (for instance in the case of major surgery, or with immune
35 deficiency) and application of apoCI in sepsis or septic

shock will lead to the prevention of death resulting from sepsis.

F. Prognosis and monitoring of sepsis or septic shock.

5   The apoCI level in blood plasma can be established in any suitable manner. Preferably, the apoCI level is determined with a sandwich Enzyme-Linked ImmunoSorbent Assay (sandwich ELISA). To this end, for instance 96-wells plates (medium-binding plates; Costar) are coated with a

10   (10,000-fold dilution of a) polyclonal antibody to human apoCI generated in goat (goat-anti human apoCI; Academy Bio-Medical Company, Houston, USA; cat. 31A-G1b). Next, human blood plasma or serum is added (100,000-300,000-fold dilution), whereby the apoCI present therein is

15   quantitatively bound by this primary antibody. The apoCI is recognized by a secondary antibody (15,000-fold dilution) which is coupled to horseradish peroxidase (HRP) (HRP-goat-anti human apoCI; Academy Bio-Medical Company, Houston, USA; cat. 31H-G1b). The HRP is subsequently quantified by

20   means of a stain reaction with 3,3',5,5'- tetramethylbenzidine (TMB) as substrate, and the stain intensity is compared with a calibration curve which is constructed with pure human apoCI (Labconsult, Brussels, Belgium; cat. A50366H).

25   Those skilled in the art, of course, know many variants of this apoCI determination. Thus, other assay techniques than ELISA can be used, other detection systems (enzymes and substrates), etc.

30

EXAMPLES

The finding that apoCI (and peptides derived
therefrom) play a protective role in Gram-negative (and
5   Gram-positive) sepsis is supported by a number of
experiments. To that purpose, use is made of:

a.   blood of septic patients;

b.   mice that do not synthesize apoCI ($apocI^{-/-}$ mice);

c.   transgenic mice that overexpress human apoCI (*APOC1*
10   mice);

d.   apoCI which has been purified from human blood.

The findings are described in the Examples given
hereinbelow.

15   Example 1: Predictive value of the apoCI level for the
chance of survival in sepsis

We have measured the apoCI levels in the blood
plasma of 17 septic patients for four weeks after admission
to the clinic. For that purpose, we used the human apoCI-
20   specific sandwich ELISA as described above under F.

The results are shown in Figure 1.

Of the 17 septic patients who were admitted to the
clinic with severe sepsis, blood samples were taken over a
period of 28 days. In the blood plasma, the contents of
25   apoCI, triglycerides (TG) and total cholesterol (TC) were
measured.

In Figure 1A the apoCI content is plotted against
time. In Figure 1B the apoCI content has been corrected for
the triglyceride content as a measure for the amount of
30   triglyceride-rich lipoproteins, and in Fig. 1C the apoCI
content has been corrected for both triglycerides and
cholesterol as a measure for the total amount of
circulating lipoproteins. A distinction was made between
the patients who survived the sepsis (closed symbols; n=9
35   ± SEM) and the patients who died from the sepsis within 30

days (open symbols; n=8 ± SEM). The dotted lines indicate
the values as found in healthy individuals.

All patients who were included in the investigation
exhibited a strongly lowered apoCI level in the blood (ca.

5   1.6 mg/dL). The patients who died from the consequences of
sepsis within four weeks, exhibited a lower apoCI level
(ca. 1.3 mg/dL) at the time of admission than did the
patients who survived the sepsis (ca. 1.9 mg/dL). While the
apoCI level virtually normalized within four weeks in

10   patients who survived the sepsis (ca. 5 mg/dL), the apoCI
level did not exhibit any rise in the patients who
eventually died from sepsis.

These data show that the plasma level of apoCI has a
predictive value for the chance of survival in sepsis.

15   Also, they underline our hypothesis that apoCI falls short
in patients who die from sepsis, and which can therefore
have a therapeutic use.

Example 2: Purified apoCI binds strongly to LPS

20   In this Example, it is demonstrated that purified
human apoCI binds strongly to LPS and that this binding is
resistant to electrophoretic forces (agarose gel
electrophoresis). It was found this involved the loss of
the naturally occurring micellary structure of LPS.

25   The results are shown in Figures 2, 3 and 4.

LPS Re595 (*Salmonella minnesota*) was radioactively
labeled with ^{125}I as described (Rensen, 1997) and incubated
(30 min at 37°C) with increasing amounts of human apoCI
which has been isolated from human blood (Tournier, 1984).

30   Next, the incubation volumes were transferred to an agarose
gel (position R_f 0), after which, for 2 hours, an
electrophoretic field was applied (so-called agarose gel
electrophoresis). After electrophoresis, the ^{125}I-LPS was
visualized by means of autoradiography. Through its

35   negative charge, LPS tends to migrate to the positive pole

(anode), which, however, is prevented in that the LPS forms
large micellary structures that cannot pass the relatively
small pores formed by the agarose matrix. However, addition
of a small amount of apoCI (only 0.5 molecules of apoCI
5   with respect to 1 molecule of LPS) already led to complete
migration of the LPS to the positive pole, which indicates
that apoCI binds to LPS thereby forming relatively small
apoCI/LPS complexes (Figure 2A).

        Also, the effect of increasing amounts of LPS on the
10   migration of a fixed amount of apoCI was determined. For
that purpose, the apoCI was visualized in the gel after
electrophoresis by means of immunoblotting with a
horseradish peroxidase-conjugated antibody directed against
human apoCI (Academy Bio-Medical Company, Houston, USA;
15   cat. 31H-G1b). The binding of LPS to apoCI led to
accelerated migration of apoCI to the positive pole, which
indicates that apoCI in effect enters into a physical
interaction with LPS (Figure 2B). The affinity of the
binding of LPS to apoCI proves to be so high that LPS
20   prevents demonstration of apoCI during immunoblotting of
the agarose gel with the apoCI-specific antibody in a dose-
dependent manner (Figure 2B).

        A particular amount of human apoCI (0.75 ng/mL) was
incubated with LPS in increasing molar ratios, after which
25   the apoCI concentration was determined with the aid of the
human apoCI-specific sandwich ELISA as described above
under F. The LPS-induced inhibition of the stain reaction
from which the apoCI concentration is read, was determined
as a percentage of the maximum staining in the absence of
30   LPS (n=2 ± SEM) (Figure 3). Again, the binding of LPS to
apoCI proved to prevent the reaction of apoCI with the
apoCI-specific antibody in a dose-dependent manner.
Addition of a 200-fold molar excess of LPS to apoCI led to
more than 40% inhibition of the staining reaction, that is,
35   a nearly twofold underestimation of the apoCI

concentration. Although apoCIII has a size (79 amino acids)
and structure (two amphipatic helices) equal to apoCI, LPS
had no effect on the quantification of concentrations of
apoCIII with the aid of a comparable apoCIII-specific
5 sandwich ELISA (Figure 3).

     Finally, the effect of apoCI on the monomerization
rate of LPS was studied. Fluorescent (fluorescein
isothiocyanate; FITC)-labeled LPS hardly exhibits
fluorescence, because the micellary structure of LPS leads
10 to the fluorescing FITC molecules being too closely packed
together, giving rise to the phenomenon of fluorescence
quenching. FITC-LPS Re595 (100 ng) was incubated with pure
human apoCI (in molar ratios 1:1, 1:5, 1:10 and 1:20),
after which the occurrence of fluorescence was followed in
15 time with the aid of a fluorimeter. ApoCI proved to cause
the increase in FITC fluorescence to increase strongly in a
dose-dependent manner, which indicates that apoCI is
capable of monomerizing the LPS (i.e. to liberate it from
the micellary form) (Figure 4A). By contrast, the
20 apolipoprotein CIII (apoCIII), having a comparable size and
structure, proved hardly effective (Figure 4B).

Example 3: Plasma apoCI binds to LPS

     We have determined the effect of the apoCI level in
25 murine plasma on monomerization of FITC-LPS as described in
Example 2. For that purpose, FITC-LPS was incubated with
plasma that had been isolated from wild-type mice,
genetically modified mice which are deficient for apoCI
($apoc1^{-/-}$), or genetically modified mice which express the
30 human apoCI hemizygote ($APOC1^{+/0}$) or homozygote ($APOC1^{+/+}$).

     The results are shown in Figure 5.

     FITC-LPS Re595 (100 ng) was incubated with plasma
(0.125%) isolated from the blood of the various mouse
types, after which the occurrence of fluorescence was
35 followed in time with the aid of a fluorimeter. The

expression of human apoCI in mouse plasma led to an apoCI-
dose dependent increase of the FITC-LPS monomerization,
which was 32% at a maximum. By contrast, the monomerization
of FITC-LPS proved to be reduced by 60% owing to the

5   absence of mouse-apoCI (n=4 ± SEM). This indicates not only
that mouse-apoCI, like human apoCI, is capable of
monomerizing LPS, but especially also that the endogenous
presence of apoCI contributes for a considerable part to
the total LPS-disaggregated capacity of mouse plasma.

10

Example 4: ApoCI extends the residence time of LPS in the
blood by preventing the uptake of LPS by the liver
     We have administered LPS intravenously to wild-type
mice in the absence or presence of purified apoCI. Addition

15   of apoCI proved to extend the residence time of LPS in the
blood strongly through increased interaction with HDL.
     The results are shown in Figure 6.
     Wild-type (C57B1/6) mice were anesthetized and, via
the lower vena cava, injected intravenously with

20   radioactively (^{125}I) labeled LPS Re595 (10 μg/kg) without,
and with, respectively, apoCI (molar ratio LPS:apoCI =
1:0.2, 1:0.5, 1:1 and 1:16) and bovine serum albumin (molar
ratio LPS:albumin = 1:16). Blood samples and bits of liver
tissue were taken at t = 2, 5, 10, 20 and 30 minutes after

25   injection. After this, the mice were sacrificed. Next, the
amounts of LPS in the blood sera and liver samples were
quantified on the basis of the amount of radioactive
radiation. Also, a serum sample was taken 30 minutes after
injection of LPS:apoCI (1:1) and subjected to agarose gel

30   electrophoresis to study the distribution of the ^{125}I-LPS
over the various lipoproteins. Lipoprotein lipids were
visualized with the aid of Sudan Black, and ^{125}I-LPS by
means of autoradiography. In Figures 6A and 6B the amounts
of ^{125}I-LPS in the liver and the blood serum, respectively,

35   are plotted as a percentage of the injected dose against

time (n=3 ± SEM). Figure 6C shows the distribution of
^{125}I-LPS over the lipoproteins. The presence of apoCI was
found to inhibit the decrease of LPS from the blood
strongly and dose-dependently. Already at a molar ratio of
5   1:1, the uptake of LPS by the liver was reduced 8-fold. The
strong binding of apoCI to LPS (see Example 2) therefore
proves to be relevant in the blood too. This led to an
extended residence time of LPS in the blood through a
strongly increased apoCI-mediated binding to HDL (Figure
10   6C), thereby improving the interaction with *inter alia*
white blood cells and eventually leading to a strongly
stimulated inflammation reaction (see Example 5).

Example 5: ApoCI stimulates the LPS-induced inflammatory
15   response in mice
We have administered LPS intravenously to wild-type
mice in the absence or presence of purified apoCI. Addition
of apoCI proved to increase the LPS-induced inflammatory
response strongly.
20   The results are shown in Figure 7.
Wild-type (C57B1/6) mice were injected, by way of the
tail vein, with LPS Re595 (25 µg/kg) without, and with,
apoCI (molar ratio LPS:apoCI = 1:5). Blood samples were
taken at t = 0, 30, 60, 90, 120 and 180 minutes after
25   injection. In the samples, subsequently, the amount of
TNFα was quantified with the aid of a mouse-TNFα specific
ELISA according to the accompanying instructions
(Immunosource, Halle, Belgium, cat. no. BMS607MST), and the
amounts of TNFα in the blood plasma were subsequently
30   plotted against time. Addition of apoCI to LPS proved to
lead to a fourfold increase of the LPS-induced TNFα level
(10.8 ± 4.5 versus 2.8 ± 1.7 ng/mL), while an equal amount
of apoCI without LPS has no TNFα-inducing effect(n=6
± SEM).
35

Example 6: ApoCI stimulates the LPS-induced inflammatory
response in humans

    We have measured the correlation between plasma apoCI
levels in patients who did or did not develop endotoxemia
5 during a heart operation with cardiopulmonary bypass, and
the TNFα level during the operation. The apoCI level
proved to be strongly positively correlated to the TNFα
response in case of a developed endotoxemia.
    The results are shown in Figure 8.
10   During a cardiopulmonary bypass, temporarily a low
oxygen pressure arises in the blood which is accompanied by
an endotoxemia through leakage of LPS from the intestines
to the blood. We investigated the correlation between the
preoperative plasma apoCI level and the plasma TNFα level
15 in 160 patients who had a heart operation involving a
cardiopulmonary bypass. Plasma samples were taken before
the operation for the purpose of measurements of lipid
parameters (apoCI, apoCIII, apoE, triglycerides and total
cholesterol). Also, plasma samples were taken after
20 inducing anesthesia (time 1), after removal of the aorta
clamp (time 2) and half an hour after termination of the
extracorporal perfusion (time 3). At times 1, 2 and 3 the
TNFα level was determined, with a distinction being made
between patients who did (LPS >5 pg/mL) or did not (LPS <5
25 pg/mL) develop endotoxemia at both time 2 and 3.
    R = Spearman's rank correlation coefficient.
    * = statistically significant (P<0.05).
    A strongly significant relation was found to exist
between the apoCI level and the TNFα level in patients who
30 during a heart operation with cardiopulmonary bypass
developed endotoxemia, but not in patients who did not
develop endotoxemia. Such a correlation is not demonstrated
for other apolipoproteins (apoCIII, apoE) or neutral lipids
(triglycerides as a measure for the amount of triglyceride-
35 rich lipoproteins, and total cholesterol as a measure for

the total amount of circulating lipoproteins), which
indicates that the effect is apoCI-specific.

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